Meyer JL (ed): The Radiation Therapy of Benign Diseases. Current Indications and Techniques. Front Radiat Ther Oncol. Basel, Karger, 2001, vol 35, pp 23–29

Cellular Effects of Radiotherapy for Benign Disease

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Cell Killing and Non-Cell-Killing Effects of Radiotherapy

In approaching the issues of radiotherapy for benign disease, what we need to know unfortunately we do not yet know. If we do not yet know the target for irradiation, there is no way to design rational improvements or to find adjunctive methods to integrate with the radiotherapy. There are some benign diseases, discussed in these chapters, in which radiotherapy effects probably can be related to cell killing. As oncologists, we are familiar with cell killing, we understand that very well, but we have difficulty understanding other changes which may not be the result of cell killing.

Cell killing is clearly important in benign tumors like meningiomas, acoustic neuromas, pituitary tumors and glomus tumors. The response of those tumors to radiation is predictable, based on their histology. They are relatively acellular, so they are cured by relatively low doses. They are also very slow to regress, or they may never regress, because most of these tumors are comprised of extracellular material that just stays there. So we can understand control of benign tumors in terms of cell killing in a dose-dependent manner.

The other area where cell killing is probably important is in immunosuppression, both for benign disease and for leukemia. In some benign disease, radiotherapy achieves its effects through the elimination of immunocytes of various categories. In leukemia it is not only suppressing the capacity of the immune system of the recipient to reject bone marrow grafts, but also eliminating leukemic cells. Beneficial effects of radiation in autoimmune disease, such as rheumatoid arthritis and multiple sclerosis may also reflect cell killing.

In contrast, cell killing may not be the major, or even a significant basis for the radiation response in thyrotoxic ophthalmopathy. When you irradiate the orbital tissues with relatively low doses, you can control the problem in about 60% of cases. The treatment (e.g. with 2,000 cGy in 200-cGy fractions) must kill most of the lymphocytes which infiltrate the ocular muscles but there is a plentiful supply of unirradiated lymphocytes that could take their place. One is just irradiating the orbits after all, and if it was purely a cell-killing phenomenon, those orbital lymphocytes could be replaced in no time at all from circulating lymphocytes. It suggests that there must be something else about the orbit, about the stroma of the orbit or the muscles, or fat, that influences the response to irradiation. And you could ask the question, why are the retro-orbital tissues the site for lymphocyte aggregation in the first place, and why are there not problems in other subcutaneous muscle or adipose tissues? There must be something that localizes the response to the retrobulbar tissues, so maybe there is some thyroid-receptor-related phenomenon influencing those tissues that we do not really know about. If we did know about them, maybe we could get more specific in the way we treat that condition.

For arteriovenous (AV) malformations, there may be a cell-killing phenomenon, but the changes that are seen are those of any injury to a blood vessel. For instance, if you look at an artery that has been tied off, or a vessel in an atrophic limb (as in someone who is paralyzed), the arteries show the same intimal hyperplasia as you see after irradiation of AV malformations. So, it may not be purely a cell-killing phenomenon that is involved in ablating AV malformations even though it is a very slow process, which is consistent with a slow rate of cell death in a very slowly proliferative system. What you see in the occlusion of the vessels is a response to radiation-induced injury.

Radiation can be successfully used to treat an AV malformation in a pig. Two years after radiation, the pathology of the radiation response is similar to the pathology one sees in vascular restenosis after angioplasty, or in a ligated peripheral artery or in the artery in an atrophic muscle. One can call it hyperplastic restenosis or atrophic endarteritis, but the pathology is similar.

In bursitis and tendinitis, radiotherapy can induce clinical responses. The radiotherapy must involve more than just the elimination of the inflammatory cells, because, as with ophthalmopathy, there are plenty more inflammatory cells circulating and available in the body to replace those that you have eliminated locally. So there must be something more than just cell killing. We do not know what it is. Later we will consider wound healing as an orchestrated phenomenon. Perhaps just by disrupting this carefully orchestrated process we can interfere with the inflammatory process and the ultimate architecture of the tissue.

Effects of Radiotherapy on Wound Healing

Radiation can greatly modify wound healing, and especially exuberant wound healing that is observed in the processes of keloid formation, heterotopic bone formation, and restenosis after angioplasty. These three processes only occur in a proportion of patients: only about 7% of patients who have hip replacements develop heterotopic bone, even fewer develop thick keloids. After angioplasty, about 40% of patients develop restenosis, which is itself a strange phenomenon.

How radiation affects keloid formation and these other excessive hyerplastic responses is a good question, for which we do not have an answer. Others have created diagrams of proposed mechanisms for these responses showing various cytokines released from cells and tissues that trigger receptors on cells in the same or different tissues. We have very limited information on which to base this modeling and, more importantly to interpret the plethora of observations. Remember that cytokines are the language that cells use to talk to one another, receptors are the ears of the cell, and the signal transduction pathways are the auditory conduction pathways to the nucleus, which is the brain of the cell. Yet we do not really understand this language now. We hear a few words. We might find a cytokine that is saying 'go', but we do not know whether it is causing the problem or whether it is just a reaction to something else that has happened further up the line of command, a link in a chain. Just identifying the fact that there are cytokines there does not establish that they are involved in the genesis of the event, or the prevention of the event, or the response to irradiation for that matter. It is an association, but not necessarily a causative one.

To consider what we might be dealing with, let us look at some observations. First, we can achieve good responses in these hypertrophic wound-healing phenomena with relatively low doses of irradiation. This is unlikely to represent a result of cell killing. For example, 7 Gy as a single dose will often prevent keloid formation, and about 12 Gy has been found to inhibit restenosis. Doses of this size may reduce cell survival to below 10%, but this would require only about 3–5 doublings to get back to where you started. So the cytotoxic effect of radiation does not explain why we get such good long-term results. This is especially true with keloids and hopefully in preventing vascular restenosis. The fact that all of these processes respond to low doses suggests a commonality of pathway. Perhaps the same processes are involved in keloid formation as in vascular restenosis and in heterotopoic bone formation.

The second observation is that the time interval between surgical injury and irradiation is very important. You can give the radiation before wounding,

or within a few days after wounding and observe a big effect. However, if you delay exposure for more than a few days, you see very little effect. There is not, however, a complete loss of effect with time. Thus, when irradiation of the breast scar (with higher doses of about 50 Gy in 2-Gy fractions) is delayed for weeks after surgery, the scars are commonly less exuberant than in unirradiated breasts. The time dependence of radiation inhibition of exuberant scarring gives some indication of what processes radiation might be affecting.

The importance of time in the effect of radiotherapy on wound healing has been explored in some work done many years ago on wound healing in the skin. Whether it is wound healing in the skin or wound healing in the arterial wall, there must be common biological pathways. The design of the experiment was to irradiate the skin, and then at some time later make an incision. Fourteen days after that, the skin was tested for tensile strength on a tensiometer. If irradiation is given 90 days before wounding the skin, or 60 days, or 40 days, or 1 day before, or at the time of wounding, there is a very significant dose-related reduction in the wound tensile strength, measured 2 weeks after the wound is inflicted. As long as you irradiate before the wounding you get a good response, but by 4, 5 or 7 days after wounding the effect of radiation on the wound tensile strength is much less than it is in the skin that has been preirradiated or irradiated shortly after wounding. When irradiation is given just before wounding, the dose response for wound healing shows a threshold of about 8 Gy, and then a dose-related decline in wound tensile strength.

What does this tell us about the mechanism? It tells us that whatever radiation is doing, it is remembered. Further, it must be remembered by cells that remain at the site of the wound, so it is not a result of an effect of circulating cells. It also must be an effect on fairly slowly proliferative cells. If they were rapidly proliferative, they would have gone through multiple divisions and expressed the radiation injury: many of the lethally injured cells would have been lost and replaced by cells that survived. So radiation injury is remembered in cells that are presumably slowly turning over and local.

We have concluded from these first observations that the radiation causes an effect on the local tissues, the target is presumably a slowly proliferative population of cells, and it is not related to migratory cells. However, if you treat the animal with half-body or total-body radiation, and evaluate the decline in wound tensile strength, the curve is shifted a long way to the left compared with local skin irradiation only. So, there must be some systemic influence despite the evidence for a major role of local cells. There must be more than one mechanism influencing wound strength in wound healing.

The radiobiology of wound healing also gives some clues about the biology of wound healing. The response curves for irradiation of the skin with a single

dose and various fractionated doses keep shifting to higher doses as you move from 2, to 4, to 8 or more fractions. The alpha/beta value for the fractionation response is 2.5 Gy, which is the sort of value to be expected from a nonproliferative tissue. Once again, local nonproliferative cells must be involved.

Just to make it more puzzling and more difficult, wound healing apparently may be temporarily enhanced by irradiation. When we irradiate mouse skin at day 4 or 7 after wounding, there is actually an increase in wound tensile strength measured at 17 or 21 days after the wounding, 14 days after radiation. This is a very puzzling turn around. The irradiation actually enhances wound healing. It is temporary; by 28 days, the 4-day and the 7-day curves are back together. If you are confused, then please join the club!

Another observation is that macrophage and lymphocyte depletion affects wound healing. If one gives total body irradiation, the depletion of circulating cells or cells distant from the site of wounding does reduce wound tensile strength. Also, antibodies to macrophages inhibit wound healing. Fetal wounds, which have less infiltrate from circulating cells, produce less scarring. The Rochester group, using a rat restenosis model, showed that radiotherapy did reduce macrophage infiltration. With the combination of systemic and local treatment, there was a greater inhibition of scarring, an effect which can also be observed after the use of steroids and nonsteroidal anti-inflammatory agents.

There are several phases of wound healing. An initial inflammatory phase lasts for 2 or 3 days, with infiltration of the usual inflammatory cells. There is a proliferative phase when the fibroblasts proliferate and undergo metaplasia to myofibroblasts. They become contractile and actin appears in the cytoplasm. Finally, the fibrous reaction undergoes remodeling, with turnover of the collagen and increasing strength of the wound. But irradiating is only really effective in reducing wound strength when it is applied in the early phases, so it has very little to do with remodeling.

Events that could be involved in the radiation response of arteries after angioplasty include any of the elements involved in thrombus formation, or it could be related to the endothelium, to the macrophages outside the medial wall, or to the muscle cells in the media. It may be related to effects on stromal cell proliferation, myofibroblast structure or function, smooth muscle cells, or osteoblasts in the case of heterotrophic bone formation. The metaplastic process may be a critical 'target' for radiation effects. In heterotrophic bone formation, the problem is that fibroblasts begin to think they are osteoblasts. In the healing of wounds in the skin, or in blood vessel walls, the fibroblasts get the idea that they are myoblasts and become contractile. So there is a metaplastic process in wound healing that may be very sensitive to radiation.

Several hypotheses have been put forward for these effects of radiation. First, adhesion molecules are important in the dynamics of wound healing and may be upregulated by radiation. However, this does not mean that they are involved in any of the things that we have been discussing. Second, many different cytokines are upregulated after radiation. TGF-β is produced and is involved in angiogenesis and macrophage recruitment, but it is not necessarily part of the mechanism by which radiation inhibits the hyperplastic wound-healing responses. The changes in cytokine production are difficult to correlate with mechanisms of injury. For example, upregulation of TNF-α, and nitric oxides that go with a macrophage type of response, is seen both early (within hours) and late (months) after irradiating the lungs of mice. So it seems to be both a direct and an indirect effect of radiation. This is happening in parallel with tissue and cellular damage, possibly to macrophages, possibly to other cells, that may take months and months to be expressed. The role of TNF-α in such a situation is not clear. It may or may not be a causal relation. Even if it is a causal relation, we do not know whether damage causes expression of TNF, or if expression of TNF causes damage.

Future Investigations

Vascular restenosis is the process of excessive neo-intimal formation. The majority of patients will not manifest this excessive proliferative response. Endothelial injury, medial injury, and thrombus deposition are processes that could conceivably be involved in this neo-intimal hyperproliferation.

Knowing the cellular and biochemical targets has practical significance for preventing restenosis. A variety of cytokines are implicated in modifying cell proliferation and migration, but investigators who want to use anti-cytokines such as anti-VEGF antibodies to prevent restenosis at present have little evidence to guide experimental initiatives. If the process is fundamentally the effect of macrophages migrating in from beyond the adventitia of the vessel wall, then one might want to continue to explore the use of gamma-emitting intravascular sources rather than beta emitters. However, if it is related only to the subintimal cells or cells in the media, then beta sources may be preferable. The targets for radiation modulation of restenosis probably have nothing to do with cells in the remodeling phase of wound healing because of the time restrictions of the effect of radiation in preventing it. Fixed tissue macrophages are a very good potential candidate. However, the fact that total body radiation or half body radiation has a big effect on the efficiency of wound healing suggests that infiltrating cells from the circulating monocytes or macrophage pools may also play a role. It may be endothelial function, a hypothesis favored

by some. The radiation may affect the functions of migration and proliferation through mechanisms other than cell death. As discussed above, something that regulates metaplasia may be involved in the radiation suppression of these hyperplastic lesions.

The tissue responses to radiotherapy for benign disease are far from well understood. Francis Bacon said that if we begin with certainties we will end in doubts, but if we begin with doubts and are patient in them, we may end up in certainties.

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